Decreased Basal Production of Nitric Oxide in Patients With Heart Disease*

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Study objectives: The pathophysiologic role of nitric oxide (NO) released in the lung is not well understood. To determine whether the production of endogenous NO is correlated with any hemodynamic parameters, we measured the amount of NO released from the lung tissue of patients with heart disease.

Methods: Twenty patients (14 with ischemic heart disease, 4 with dilated cardiomyopathy, and 2 with mitral stenosis) and 16 normal control subjects were enrolled in the study. We measured exhaled air samples by using a method developed in our laboratory. The NO release rate from the lungs was calculated from the amount of exhaled NO and the duration of the exhalation.

Results: The rate of NO release was significantly lower in the patients with moderate-to-severe heart failure (New York Heart Association [NYHA] II or III) than in those with mild heart failure (NYHA I) or in normal control subjects. The rate of NO release was positively correlated with the cardiac index (r=0.50, p<0.05), and was negatively correlated with either the systemic (r=−0.58, p<0.01) or pulmonary vascular resistance (r=−0.45, p<0.05). In the patients with moderate-to-severe heart failure, the amount of NO released and the oxygen tension in the pulmonary artery were significantly lower compared with those parameters in patients with mild heart failure.

Conclusions: Results suggest that the basal production of endogenous NO in the lung tissue of patients with heart failure is impaired, perhaps leading to the elevated pulmonary vascular tone seen in patients with moderate-to-severe heart failure. *(CHEST 1998; 113:317-22)*

Key words: cardiac index; chemiluminescence analyzer; hypoxic pulmonary vasoconstriction; vascular resistance

Abbreviations: L-NMMA=N^G^-monomethyl-L-arginine; NO=nitric oxide; NYHA=New York Heart Association; ppb=parts per billion

Heart failure with a reduction in cardiac output is accompanied by increased systemic and pulmonary vascular resistances. These abnormalities in vasomotor tone have been explained by known neurogenic and humoral stimuli, such as an activation in both the sympathetic and the renin-angiotensin systems.1 Recent advances in vascular biology designated nitric oxide (NO) from the vascular endothelium as an important controlling factor of vascular tone.2 Several studies have shown that the vasodilatory response or the blood flow response to acetylcholine is blunted in the peripheral resistance vessels in heart failure, indicating an impairment in receptor-mediated NO release from the endothelium.3,7 In contrast, the peripheral vasoconstrictor response to N^G^-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthesis, is exaggerated, suggesting that the basal release of NO from the endothelium of resistance vessels is enhanced.4 The pulmonary vasoconstricting response to L-NMMA has been studied in both normal humans and patients with heart failure.8-10 The results suggested that the basal release of NO from pulmonary vessels is also exaggerated in the patients. Thus, it is still not clear whether NO release from systemic and pulmonary vascular endothelia is enhanced or inhibited in heart failure.

To clarify the actual contribution of NO to the vascular tone in heart failure, a direct estimation of NO production from the vasculature has an advantage over the estimation of NO activity in response to acetylcholine or L-NMMA. Recently, the presence of endogenous NO in the exhaled breath of humans has been demonstrated.11 Although various types of

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cells are reportedly sources of NO in the lung. Additional reports suggest that pulmonary endothelium-derived NO enters the lungs in amounts sufficient to allow its measurement in exhaled air. It is therefore appropriate to use the amount of NO measured in expired air as an indicator of the endogenously produced NO levels in the pulmonary vasculature. We recently developed a method for assessing the NO level in exhaled air.

We hypothesized that the regional production of NO from the pulmonary vascular bed would contribute to the vascular tone of the pulmonary circulation. The present study assessed whether the production of NO would be correlated with hemodynamic parameters. We also estimated the involvement of the pulmonary-derived NO in the hemodynamic alterations characteristic of heart failure.

**Materials and Methods**

**Subjects**

Twenty patients with heart disease and 16 normal control subjects were enrolled in this study of Japanese subjects. The patients consisted of 11 men and 9 women aged 57±12 years (range, 40 to 77 years). Fourteen patients had ischemic heart disease, four had dilated cardiomyopathy, and two had mitral stenosis. The 20 patients were classified according to the New York Heart Association (NYHA) as follows: class I (n=14), class II (n=3), and class III (n=3). The control subjects were 14 men and 2 women aged 41±11 years (range, 28 to 55 years).

In the patients who underwent pulmonary function testing (Chestac-55V; Chest Ml, Tokyo, Japan), the FVC, expressed as a percentage of reference value, was 98.7±12.7% (range, 71.6 to 122.8%), and the FEV1, expressed as a percentage of the forced expiratory volume, was 79.0±5.4% (range, 68.0 to 87.5%). The following drugs were being administered: calcium antagonist (11), nitrate-containing agent (12), angiotensin-converting enzyme inhibitor (6), furosemide (6), and digoxin (4). None of the normal subjects were receiving any medication. All subjects in this study lacked evidence of active infection or of respiratory disease. The mean body surface area was 1.63±0.16 m2 (range, 1.23 to 1.92 m2) in the patients and 1.75±0.15 m2 (range, 1.43 to 1.99 m2) in the control subjects. Seven subjects were smokers in each group. Any medications being taken by the patients were interrupted >12h before the study. Alcohol, caffeine, and cigarettes were prohibited in the 12h before as well as during the study. The study protocol was approved by the Institutional Committee on Human Research of Gunma University Hospital. Informed consent for participation was obtained from each subject.

**Study Procedures**

All subjects were allowed to sit and breathe normally for 10 min prior to the collection of exhaled air samples. After the air sampling, right-sided heart catheterization and arterial cannulation were performed in all patients with heart disease as described below.

Right-sided heart catheterization was performed using a 7F Swan-Ganz catheter (Baxter; Irvine, Calif) inserted percutaneously through the right femoral vein and advanced to the pulmonary artery. Cardiac output was determined by the thermodilution technique in which 10 mL of saline solution was injected and monitored by using a cardiac output computer (COM-1; Edwards; Irvine, Calif). More than three measurements were averaged to obtain a mean cardiac output. A 5F conductance catheter was also inserted percutaneously through a 6F sheath and advanced from the femoral artery to the left ventricle. Blood was sampled at the ascending aorta and at the pulmonary artery to measure oxygen tension with a blood gas analyzer (model 278; Ciba-Corning; Boston).

**Collection of Exhaled Air**

Samples of exhaled air, the analysis of NO gas, and the determination of the rate of NO release were obtained by our previously described method. In brief, by using a nose clip, air inspired through the mouth was exhaled through the mouth into a 6-L bag made of polyvinyl fluoride film (Tedlar bag; Inchi, Tokyo, Japan). A Teflon column (15×50 mm) was inserted between the mouthpiece and the bag. The column was packed with 6.7 g of silica gel (particle size, 1.7 to 4.0 mm; Kanto Chemical; Tokyo, Japan). This column was used to dry the exhaled air sufficiently to prevent the condensation of vapor on the walls of the bag. NO was not adsorbed by the silica gel and was stable in the bag for several hours if the sample was dry and not exposed to light. The study was performed only when the concentration of NO in the room air was <3 parts per billion (ppb). The average room air concentration of NO was 1.2±0.6 ppb (0.6 to 2.8 ppb) immediately before initiation of each measurement on the days of the study.

**Analysis of NO and Determination of NO Release Rate**

A chemiluminescence analyzer (model GLN-32; Denki-Kagaku-Keiki; Tokyo, Japan) was used to measure NO concentrations. The flow rate for sampling was 500 mL/min. The limit of detection for NO was 0.2 ppb. The coefficient of variation was 2% at 20 ppb of NO.

After making a full inspiratory effort, each subject was instructed to exhale air at six flow rates that were determined arbitrarily by each individual. These flow rates ranged from very fast to very slow. Air volumes in the six efforts corresponded to the individual’s vital capacity. The six samples were collected in separate bags. The time from the start to the end of each sampling was measured with a stopwatch. Sample volumes were determined on a chart recorder (model 056; Hitachi; Tokyo, Japan).

The concentration of NO in exhaled air was positively correlated with the duration of exhalation. By using the slope of a simple linear regression model applied between the concentration of gas and the duration of exhalation, the rate of NO release for each subject was calculated as follows: release rate of NO (pmol/s)=slope (ppb/s)×average of six sample volumes (L)×1/V (L−1)×104, where V corresponds to the volume of 1 mole of dry air at ambient temperature, and is 24.5 L at 25°C and 760 mm Hg. The release rate was constant (35±4 pmol/s) in one control subject who repeated the protocol 10 times over a 2-week period.
Data are expressed as mean±SD. An unpaired two-tailed Student’s t test or Welch’s test was used to analyze the differences between the control values and those from the patients with heart failure, and the differences between the two subgroups of patients. The χ² test and a Fisher’s probability test were used to compare proportions. The coefficient of correlation (r) was computed with a software package (Statview SE; Abacus Concepts, Inc; Berkeley, Calif). A level of p<0.05 was accepted as statistically significant.

RESULTS

The mean (±SD) release rate of NO, which was normalized to the body surface area, was slightly lower (p=0.07) in the patients with cardiac disease (19.0±8.3 pmol/s/m², n=20) than in the normal control subjects (24.4±9.3 pmol/m², n=16). The rate of NO release was significantly lower in those patients with heart failure classified as NYHA II or III (11.3±4.6 pmol/s/m², n=6) as compared with those with mild heart failure classified as NYHA I (22.1±7.4 pmol/s/m², n=14) or with the normal control subjects (Fig 1). The rate of NO release in the patients with heart disease was positively correlated with the cardiac index (r=0.50, p<0.05) (Fig 2), and was negatively correlated with the systemic vascular resistance (r=−0.58, p<0.01) and pulmonary vascular resistance (r=−0.45, p<0.05) (Fig 3).

The patients with heart disease were subdivided into two groups, according to severity: a mild heart failure group and a moderate-to-severe heart failure group (Table 1). The moderate-to-severe heart failure group exhaled a significantly smaller (p<0.01) mean amount of NO than did the mild heart failure group. The mean pulmonary vascular resistance was significantly higher in the mild vs the moderate-to-severe groups (p<0.01). The mean pulmonary artery pressure did not significantly differ between these two groups. The oxygen tension in the pulmonary artery blood was significantly lower in the mild heart failure patients as compared with the moderate-to-severe group (p<0.05), but tended to be reduced in aortic blood (Table 2). The two groups of patients showed no significant difference in age, sex, smoking habit, drug regimen, body surface area, FVC, or FEV₁.

DISCUSSION

The present study showed that the amount of NO released from the lungs of patients with impaired cardiac function was decreased relative to those with normal cardiac function. Prior studies have yielded conflicting information about the involvement of NO in regulating the basal pulmonary vascular tone. In a rat model of chronic heart failure, the basal endothelium-derived relaxing factor activity was impaired in the pulmonary artery. Habib et al have shown that L-NMMA infusion increases the pulmonary vascular resistances in patients with heart failure, suggesting an enhanced basal production of NO. However, their observation does not establish that NO originating basally in pulmonary vasculature is enhanced, since the vasoconstrictor responses with L-NMMA administration may be modified by mechanisms other than simple inhibition of NO synthe-
sis. Our result shows a reduced basal production of NO in the pulmonary vasculature in patients with heart failure.

Table 1—Demographic Characteristics for Patients with Heart Disease*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mild HF</th>
<th>Moderate-to-Severe HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>56±12</td>
<td>62±10</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>9/5</td>
<td>2/4</td>
</tr>
<tr>
<td>Etiology of cardiac disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mitral stenosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Associated disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mild HF=mild heart failure (NYHA I); moderate-to-severe HF=moderate-to-severe heart failure (NYHA II or III).

Table 2—Exhaled NO and Hemodynamic Data for Patients With Heart Disease*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mild HF</th>
<th>Moderate-to-Severe HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO release, pmol/s•m²</td>
<td>22±1.7</td>
<td>11±3±4.61</td>
</tr>
<tr>
<td>Cardiac index, L/min•m²</td>
<td>2.95±0.60</td>
<td>2.2±0.23</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyne•cm⁻⁵</td>
<td>94±32</td>
<td>138±38</td>
</tr>
<tr>
<td>Mean pulmonary arterial pressure, mm Hg</td>
<td>17±4</td>
<td>18±3</td>
</tr>
<tr>
<td>Pulmonary wedge pressure, mm Hg</td>
<td>11±3</td>
<td>13±3</td>
</tr>
<tr>
<td>Pulmonary artery Po₂, mm Hg</td>
<td>39±3</td>
<td>32±61</td>
</tr>
<tr>
<td>Aorta Po₂, mm Hg</td>
<td>84±12</td>
<td>78±14</td>
</tr>
</tbody>
</table>

*See Table 1 footnote for column head explanation.

Our finding that the production of NO is decreased in patients with heart failure is controversial in light of the observation of Winlaw et al., who found that the levels of plasma nitrate, a stable end product of NO production, are higher in patients with congestive heart failure than in normal subjects. One possible reason for this may be the reduction in renal function that accompanies cardiac dysfunction. In cases associated with alterations in renal function and extracellular fluid volume, caution should be exercised when increases in plasma nitrate levels are evaluated as an index of NO formation. It has also been shown that in rabbits, NO levels generated from sites with an access to the alveolar space do not parallel the NO levels released intravascularly. Another reason for the discrepancy between our findings and those of Winlaw et al. may be the use of a different sampling site for measurement of NO production.

We found that NO production in the lung was negatively correlated with pulmonary vascular resistance. In addition, a relationship between this lower production of NO and higher pulmonary vascular resistance was observed in patients with impaired cardiac function. These findings suggest that a diminished NO production in the pulmonary vasculature is related to the pulmonary vasoconstriction observed in patients with an impaired cardiac output. Other local endothelium-dependent and endothelium-independent mechanisms, including prosta-cyclin activity and endothelin formation, are also important in regulating the pulmonary vascular resistance. Although interactions between these mechanisms and the production of NO must be considered, no such interactions were assessed in the present study. Nevertheless, given the significant relationship between the production of NO and
pulmonary vascular resistance, a decrease in NO production was partly involved in elevating the pulmonary vascular tone.

Interestingly, we have observed also that NO production in the lung was negatively well correlated with systemic vascular resistance. This result suggests that the amount of NO release in the lung may parallel the basal release of NO from the peripheral resistance vessels. There is evidence that the production of NO from the aorta or the coronary microvessels is reduced in dogs with heart failure, indicating the decreased basal production of NO.25,26 If it is true in human heart failure that a decreased production of NO in the peripheral resistance vessels occurs in basal conditions, the amount of NO released from the lungs would be a useful indicator that reflects the severity of heart failure.

Pulmonary vasoconstriction during hypoxia is essential to the maintenance of vasoregulation in the lung, serving to match ventilation and perfusion and to preserve arterial oxygenation.27 While NO may serve as an endogenous modulator of the hypoxic pressor response, it is unclear whether an increased synthesis of NO during hypoxia would reduce the vasoconstrictor response, or whether a reduction in NO synthesis during hypoxia would contribute to the pressor response. However, it has been reported that hypoxia resulted in pulmonary vasoconstriction in rats via a reduction in NO levels.28 Conversely, other studies have provided evidence for an elevated compensatory release of NO in hypoxic rats29,30 and dogs.31 In chronically hypoxemic patients with obstructive pulmonary disease, it is speculated that chronic hypoxemia leads to the impairment of endothelium-derived relaxing factor release.32 It has been demonstrated in human vascular endothelial cells that hypoxia inhibits the expression of constitutive NO synthase via transcriptional and posttranscriptional mechanisms.33 Hypoxia leads to a sharp drop in exhaled levels of NO in animal models.13,21 These phenomena may explain the lowered release of NO in the lungs in our group of patients with low cardiac outputs. An increase in blood flow enhances the receptor-stimulated production and release of NO.34 Patients with congestive heart failure exhibit a slow pulmonary blood flow, particularly in regions of the lung with an elevated pulmonary venous pressure.35 A decrease in blood flow may be another mechanism of the reduction in NO release.

We divided our patients into two groups with differing severities of heart failure, a mild heart failure group and a moderate-to-severe heart failure group. While the level of NO production was lower in the moderate-to-severe heart failure patients, this difference may be attributable to an associated disease, the drug regimen, or possibly to habitual smoking. Diabetes,36 hypertension,37 and hypercholesterolemia,38 for example, each impair the vascular relaxation that is dependent on the endothelium. In the patients under study, such associated diseases occurred more often in the group with mild heart failure, which suggests that associated disease did not influence NO production. Various drugs are reported to affect NO production.39,40 However, the proportion of patients who were taking such medications was similar in the two subgroups. Habitual smokers were similarly distributed between the two subgroups.

We found that the concentration of NO present in exhaled air was reduced in patients with a low cardiac output. This reduction in NO levels may indicate a diminished basal production of NO in the pulmonary vasculature that may lead to pulmonary vasoconstriction. This finding may explain in part why patients with heart failure exhibit an increased vasoconstriction of their pulmonary blood vessels.

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